



Tulane University Medical Center

NCC2-5011

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Ms. Adrienne Erwin
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Moffett Field, Ca. 94035-1000

February 8, 1999

Dear Ms. Erwin:

This is the final report on Joint Research Interchange (NCC2-5011) "Construction of a Specialized Cloning Strain of *E. coli* for the Nitrate Reductase Genes of *Haloferax denitrificans*." Originally the award was 11/1/93-10/31/95, but there were no-cost extensions made, because of a year Sabbatical at the Pasteur Institute in Paris and other leaves of 3 months each at the Pasteur institute, during which work could not be done on this project, which extended the closing date to 10/30/98.

I shall begin with a brief discussion of the goal and rationale of the project as originally envisioned by the collaborative investigators, myself and Dr. Lawrence Hochstein.

Dr. Hochstein worked for many years on the biochemistry and enzymology of halophilic bacteria. At the point that this project commenced he was very interested in trying to clone the nitrate reductase gene(s) of *Haloferax denitrificans*. Attendant to this eventual pursuit was the need to satisfy certain prerequisites specific to cloning this gene(s) of this organism. I will not enumerate all of those considerations, but simply point out that on carefully evaluating the goal of the project the need for a specialized cloning strain became evident. This was the origin of the collaborative project for which this is the final report.

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It was reasonably clear that the cloning strain would have to have certain specific characteristics over and above what is commonly the case in the readily available cloning strains. This necessitated constructing this strain. Additionally, it seemed at the time that the only feasible approach to the cloning operation when undertaken would be a two tiered system. The strain to be constructed would in the first stage, as is commonly the case in cloning operations, be designed simply to maximize selection for clones with inserts of halophile DNA. Then in the second stage it would be designed to attempt to complement strains of *E. coli* possessing insertional inactivation (a functional deletion) of the nitrate reductase genes, with the putative cloned nitrate reductase gene(s) of *Haloferax denitrificans*.

I am happy to report that we have succeeded in constructing just such a strain and therefore the goal of the project has been successfully completed. Unfortunately, Dr. Hochstein retired last year and I have now retired as well, therefore the cloning project cannot be pursued by either of us.

The specialized cloning strain has the following characteristics:

1. It has the lac selection system common to many cloning systems. The system consists of a deletion of the lac genes on the chromosome. The strain also contains F'(Δ lacZM15). The sequences coding for the Δ lacZM15 complementing lac fragments must be introduced on the cloning plasmid and should contain several cloning sites within those sequences. This permits the blue-white colony differentiation on x-gal plates for clones containing insertions of the DNA one wants to clone.

2. There is an insertional inactivation of one of the nitrate reductase genes on the chromosome to allow for a colorimetric test of functional complementation, distinguishing between nitrate reductase negative and positive organisms by differences in colony color. This permits the rapid screening of clones of DNA sequences

representing the whole chromosome of *Haloferax denitrificans* for the nitrate reductase gene(s).

3. This strain has an unusual array of antibiotic resistances which makes it essentially foolproof against any conceivable contaminants. It is resistant to streptomycin 200µg/ml, ampicillin 100ug/µl (if pUC cloning plasmids or their derivatives are used. Also one could use other cloning plasmids with other antibiotic resistances provided they contain sequences complementing the *ΔlacZM15* complementing sequences within which are the cloning sites, and tetracycline 100µg/ml. Of particular note is the tetracyclin resistance. The normal resistance level for most bacteria to tetracycline is 10-15 µg/ml. A resistance level of 100µg/ml virtually assures that nothing but this specialized cloning strain will grow on the selection plates. With these characteristics the strain is indeed specialized for the intended goal.

However, as previously mentioned, with the retirement of Dr. Hochstein prior to the last no-cost extension and now my retirement, this will conclude this collaborative research project.

This strain will be made available in accordance with your instruction.

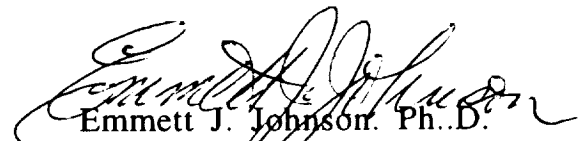
In addition I will be at the Pasteur Institute beginning March 2. My e-mail address there is:

ejohnson@pasteur.fr

My mailing address is:

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Sincerely,


Emmett J. Johnson, Ph.D.
Professor Emeritus

cc: Dr. Andrew Pohorille